https://doi.org/10.48047/AFJBS.6.13.2024. 631-649

African Journal of Biological Sciences

IMPACT OF PROCESSING ON COCOA BEANS: FUNCTIONAL COMPOUNDS, ANTIOXIDANTS, AND SENSORY CHARACTERISTICS

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Abstract

Changes in functional compounds and antioxidant capacity during processing were investigated in cocoa beans, and the sensory profile of cocoa paste was determined. 97 cocoa samples were collected from Tingo María (TM), 17 from San Alejandro (SA) and 21 from Curimana (CU), in Peru. The content of total phenolic compounds in fresh grains differed between areas. The highest antioxidant capacity was found in fresh grains GCZfres, CM4fres and OC2fres. The GCZ9li cocoa paste sample had the lowest anthocyanin content. Antioxidant capacity was mainly influenced by phenols, followed by anthocyanins and reducing sugars. OC2 cocoa paste had a distinctive cocoa flavor, while GCZ9 had an intense cocoa flavor with fruity notes and marked acidity, and CMA4 had a similar but less intense flavor than OC2.

Keywords: Phenols, anthocyanins, reducing sugars, conching, flavor.

Article History

Volume 6, Issue 13, 2024

Received: 18June 2024

Accepted: 02July 2024

doi:*10.48047/AFJBS.6.13.2024.* 631-649

1. Introduction

Cocoa is native to the tropical forests of Central and South America (Mata, 2018). The occurrence of Theobroma cacao in Perú has been documented in the departments of Cajamarca, Cusco, Huánuco, Junín, Loreto, Madre de Dios, Piura, San Martin, Ayacucho, Pasco, and Ucayali (Dostert et al., 2012). There are three major genetic groups of cacaos: Criollo, Outsiders, and Trinity (Żyżelewicz et al., 2018). No clutch, contemporary molecular genetic studies have confirmed the wide genetic diversity of the species (Diaz y Pinoargote, 2012).

Cocoa in all its forms supports brain health, acts as a good source of antioxidants, regulates the level of cholesterol in the blood, helps to reduce obesity, regulates cardiovascular health, and prevents cancer (Tardzenyuy et al., 2020). Roasting causes a decrease in the wealth of phenols, but not antioxidant activity (Suazo, 2012). Phenolic compounds or phenols are one of the largest groups of secondary metabolites and are ubiquitously distributed among plant species, with more than 8,000 chemical structures so far reported (Arias et al., 2018), which are characterized by having in its chemical structure at least one aromatic ring attached to one or more hydroxyl groups and are frequently derivatives as esters, ethers, and glycosides (Gil, 2012). The health benefits of cocoa phenols, as reported in recent studies, have increased the interest in obtaining products from cocoa beans, not only with high polyphenol but also with high flavan-3-ol content. The main flavan-3-ol compounds present in cocoa are the monomers catechin and epicatechin, and the dimer procyanidin B2 (Schinella et al., 2010; Ioannone et al., 2015).

An antioxidant is a substance capable of neutralizing the oxidizing action of free radicals, releasing electrons in the blood are captured by free radicals, maintaining its stability (Avello y Suwalski, 2006). The antioxidant properties of cocoa have been studied extensively in recent years. The main compounds of cocoa and chocolate, which contribute to human health, are phenols that act as antioxidants and have potential anti-inflammatory, cardioprotective, antihepatotoxic, antibacterial, antiviral, antiallergenic, and anticarcinogenic properties (Ackar et al., 2013; Ooi et al., 2020). Cocoa flavonoids have been demonstrated to influence several important biological functions in vitro and in vivo by their free radical scavenging ability or through the regulation of signal transduction pathways to stimulate apoptosis and to inhibit inflammation, cellular proliferation, angiogenesis, and metastasis (Martin et al., 2013).

Anthocyanins are natural pigments that are responsible for blue, purple, violet and red fruits, vegetables, cereals, tubers, grains, and flowers, which are a class of flavonoids with the

highest antioxidant activity. Anthocyanins are synthesized by the route of phenylpropanoids (Ramos et al, 2010).

The cocoa bean fermentation process is vital for the production of quality chocolate and is carried out in different ways in different regions (John et al., 2019). During the fermentation process is observed a decrease in the content of reducing and total sugars in the pulp fraction's head. The cotyledon has been found to reduce these reducing sugars during fermentation (Portillo et al, 2007).

Chocolate is a key ingredient in many types of food and is ranked as one of the most favorite flavors worldwide. It is derived from the beans of the cocoa tree fruit through several processes. The cocoa liquor, which is obtained, is the base raw material for the chocolate industry (Di Carro et al., 2015). Cocoa composition and fatty acid (FA) profile varied depending on geographical origin, whereas in chocolates, only carbohydrates and fat content varied significantly due to the effect of origin, but no significant effect was observed for processing conditions. Ecuadorian chocolate showed a healthier FA profile with higher amounts of unsaturated FA and lower amounts of saturated FA than Ghanaian chocolate (Torres-Moreno et al., 2015). It was found that higher concentrations of theobromine, caffeine, catechin, and epicatechin are present in fresh cocoa beans. The catechin and epicatechin contents decreased during fermentation, drying, and cocoa liquor processing. Moreover, cocoa variety influences cocoa liquor quality (Peláez et al., 2016).

As it could be seen in the background, there are changes in the contents of phenols, anthocyanins, reducing sugars, and antioxidant capacity during the processing of cocoa beans, until paste is obtained, these variations are affected by the place of origin, variety of cocoa, and other aspects. Thus, this study aimed to determine the changes in functional compounds, antioxidant capacity, and sensory profile of paste, in the processing of cocoa beans from three geographic areas. These variables have not yet been adequately investigated in cocoa processing.

2. Materials and methods

2.1. Cocoa beans

The cocoa pods were collected from trees in the cooperative's geographical areas of influence (Figura 1), which includes the areas of Tingo María (Pumahuasi, Mapresa, Valle Hermoso, Huayhuante, Pendencia, Frontera, Antonio Mansilla, Huayhuantillo, San José de Pucate), at an altitude of 660 m.a.s.l. at 09° 17’ 08” from south latitude, at 75° 59’ 52” from west latitude, San Alejandro (Huipoca, New Bellavista, New Ucayali), at an altitude of 217 m.a.s.l.

at 08° 49' 44" from South Latitude, at 75° 12' 44" from west latitude, and Curimana (San Jose, Pedregal), at 08º 27' 51" from south latitude, 75º 09' 03" from west latitude, samples of cocoa beans were obtained from selected trees with better features, according to information provided by farmers. The collection was made with the support of the technical team of the cooperative, and 3 to 5 pods per tree were collected.

2.2. Reagents

Gallic acid (C7H6O5) 98.1% Sigma Aldrich, Folin-Ciocalteu phenol reagent, Sigma Aldrich, 2N; sodium carbonate (Na2CO3) lSO -Scharlau, pa; 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Sigma Aldrich; Induquimica methanol 99% pure; Induquimica chloroform, distilled deionized water (ddH2O), dinitro salicylic acid (DNS), lead acetate Sigma Aldrich, sodium hydroxide; purity 99.5% Sigma; buffer pH 1 and pH 4.5.

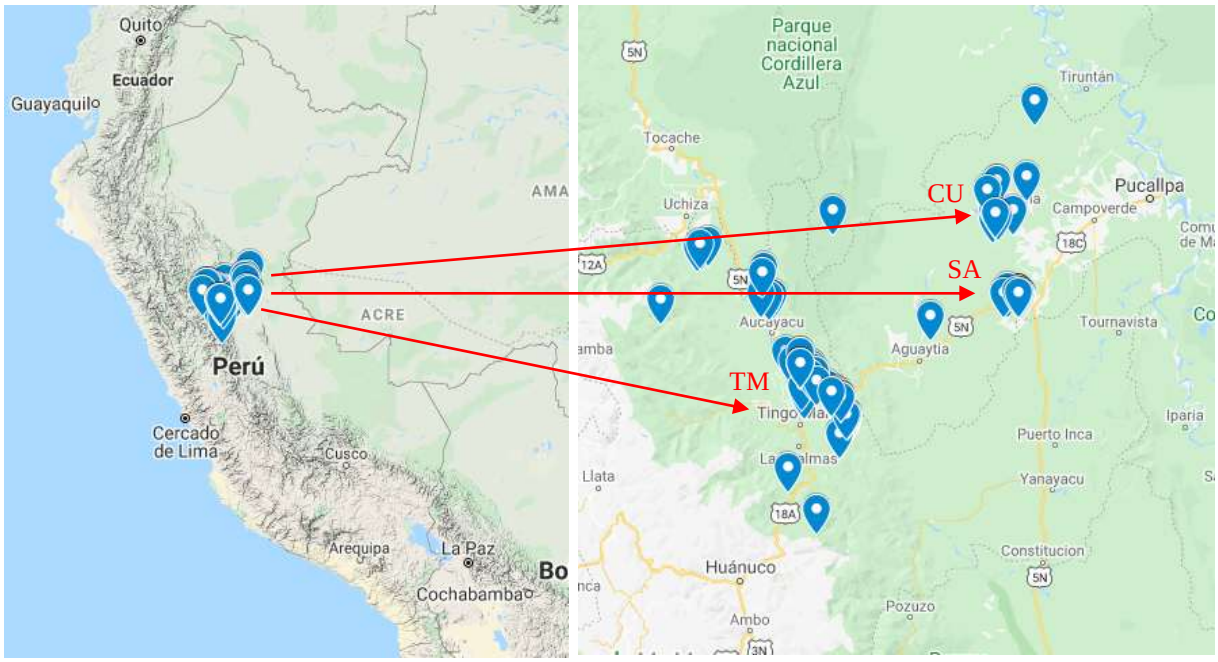


Figure 1. Geographical location of sampled trees areas of Tingo María (TM), San Alejandro (SA), and Curimana (CU).

2.3. Methods of analysis

2.3.1. Determination of moisture

The moisture content in the fresh and dry cocoa beans was determined using the gravimetric method number 931.04 (AOAC, 1995).

2.3.2. Quantification of total phenols

It was determined spectrophotometrically according to the Follin-Ciocalteau method, expressed as gallic acid equivalents (Symonowicz et al., 2012; Sultana et al., 2012).

2.3.3. Quantification of anthocyanins

It was determined by the differential pH method (Poo, 2005; Symonowicz et al., 2012).

2.3.4. Quantification of reducing sugars

The dinitrosalicylic acid reagent, developed for the determination of reducing sugars, is composed of dinitrosalicylic acid, Rochelle's salt, phenol, sodium bisulfite, and sodium hydroxide. Rochelle salt was introduced to prevent the reagent from dissolving oxygen, and phenol, to increase the amount of color produced, and bisulfite, to stabilize the color obtained in the presence of phenol. Alkali is necessary for the reducing action of glucose on dinitrosalicylic acid (Miller, 1959; Gusakov et al, 2011).

2.3.5. Determination of antioxidant capacity (IC_{50}) of DPPH

The ability to inhibit 2,2-diphenyl-1-picrythydrazyl radical (DPPH) was performed using the UV-Visible spectrophotometer method at 510 nm (Brand-Williams et al., 1995).

2.3.6. For the sensory profile

An affective analysis "measure of satisfaction" was used. With this analysis, the acceptability of cocoa paste was determined by the consumer, with the participation of trained panelists, members of the cooperative, a scale of 0 to 10 was used (García et al., 2017).

2.4. Experimental methodology

2.4.1. Sample pretreatment

Figure 2 shows the flow chart of cocoa bean processing and the times at which samples were taken for analysis. First, the fruits are harvested, cut into two parts; cocoa beans are extracted, fermented, and dried. With dry beans, cocoa paste was prepared. Samples were taken for analysis, as fresh grain, dry fermented beans, and cocoa paste; these samples according to the flow of Figure 2 were dried, packed, sealed, and stored according to sample type.

Drying of fresh and fermented grains was performed in an oven at 35 °C / 8 h and 65 °C / 18 h to a moisture content of 7%. The packaging and sealing of the dried samples were conducted in HDPE bags. Storage was held in frozen storage at -20 °C until further analysis. Fermentation was carried out with 1 kg of grains, which were placed in a nylon mesh, which allowed to maintain "the inserted sample (IS)" within the drawer fermentation with other grains, as an independent sample, but exposed to the same conditions as the rest of the grains of the box. The IS was placed on top of the box to a depth of 30 cm. Fermentation was carried out in boxes with 200 kg of cocoa baba. The total fermentation time was 120 h.

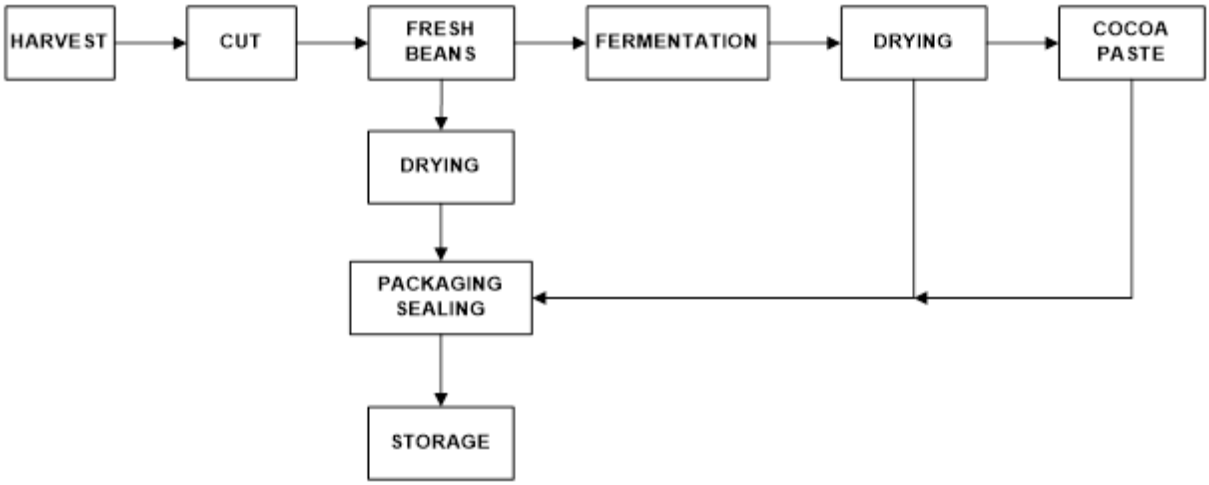


Figure 2. Flowchart showing the processing of cocoa beans and sampling.

For the preparation of the cocoa paste dry fermented grain samples were used. This process was conducted on-site quality control laboratory of the cooperative, which was implemented. For cocoa paste, the following was considered: Selection (beans fermented dry were screened using sieves of meshes of a centimeter hole, allowing the passage of smaller grains, retaining the highest caliber (NTP 208 040 2008)), heavy (it weight 750 g of fermented grains dry), toast (preheat the oven to 150 °C / 4 min) was performed. Roasting of cocoa beans was carried out at 115 °C for 20 min, grinding (cooled and crushed grains, until loose nibs husk), descaling (grain ground by the shelling is passed, until free from husk nibs), conching (the nibs crushed roasted cocoa beans, were reduced in size, performed at 49 °C for 9 h, to obtain cocoa paste). Later, the paste was retired molding and refrigerated. After 8 h, it was unmolded, placed in polyethylene high density, samples were identified, putting on the label: production date, type of cocoa, committee sources, and sample code. The samples were stored refrigerated until evaluation.

2.4.2. Total phenol content in fresh cocoa beans by geographic area

Total phenol content was found in each of the samples of fresh cocoa beans, of all trees assessed, considering the production areas of Tingo Maria (TM), with 97 trees; San Alejandro (SA), with 17 trees; Curimana (CU), with 21 trees. Hydroalcoholic extracts of cocoa were prepared at a concentration of 100 mg/mL. To this, 10 g of defatted sample was weighed, transferred to a glass jar amber, and then added 100 mL of alcohol solution (50:50 v/v), capped tightly and macerated for 24 h, filtered, and stored in amber vials at -18 °C. At this stage were selected three samples with the highest phenol content, taking one sample per area under study. These selected samples were used in the following steps.

2.4.3. Obtaining cocoa paste and variation of functional compounds

In this stage, the selected and conditioned samples were used, and one sample was selected for each geographical area in order to evaluate the variation in the contents of total phenols, anthocyanins, and reducing sugars during the process of obtaining cocoa paste. The samples were fresh beans, dry fermented beans, and cocoa paste.

2.4.4. Antioxidant capacity

Cocoa beans from selected trees were used for fermented beans, dry beans, and cocoa paste. In this process, the variation in the antioxidant capacity was evaluated using the DPPH radical, the IC_{50} inhibition coefficient was determined, and the degree of correlation between the antioxidant capacity and the content of total phenols, anthocyanin, and reducing sugar content was also established. All analyses were performed in triplicates.

2.4.5. Sensory profile of paste

The sensory evaluation of cocoa paste was to taste three samples of paste, using a trained panel of the cooperative. It was performed by applying a scale of 0-10 (0 is absent, 1-2 low, 3-5 medium, 6-8 high, 9-10 very high and strong). The tasting panel members evaluated the basic tastes (acidity, bitterness, astringency, and sweet), specific (cocoa, floral, fruity, and nutty), and acquired (moldy, and raw/green) (García et al., 2017).

2.4.6. Statistical analysis

In the statistical analysis of the total phenol content, a complete randomized design was used (Daza, 2006; López, 2008), Statgraphic Centurion 15.1 V software was used. The results of the variation of the antioxidant capacity and the correlation with the contents of total phenols, anthocyanins, and reducing sugars were statistically evaluated using the multivariate method of principal component analysis (PCA) (Mesa et al., 2018). Statistical analysis was performed using The Unscrambler® X software, version 10.4. ©2016. CAMO Software AS (Esbensen and Swarbrick, 2017). An analysis of variance and the Kruskal Wallis test were used to evaluate the results of the sensory attributes (Romaina, 2012).

3. Results and discussion

3.1. Total phenol content in fresh cocoa beans by geographic area

Figure 3 shows the total phenols content found in each of the samples of fresh cocoa beans, of all trees assessed, considering the production areas of Tingo Maria (TM), with 97 trees; San Alejandro (SA), with 17 trees; Curimana (CU), with 21 trees. The samples that had high total phenol contents were the OC2 sample showed 8.8 ± 0.1 g EAG / 100 g of grain from TM zone; GCZ9 sample 7.0 ± 0.02 g EAG / 100 g of grain from SA zone; CMA4 sample 6.1 ± 0.02 g EAG / 100 g grain from the CU zone. These results were similar to those reported in a

study conducted in Colombia (Carrillo et al., 2014), which reported a variation in the phenol content between 4.7 g / 100 g to 7.0 g / 100g cocoa beans.

Statistical analysis of total phenol content in fresh cocoa beans by area of production, indicating that there was a statistical difference ($p \leq 0.01$) in the total phenol content between samples. According to the Tukey test, they highlighted the samples indicated by their higher content of phenols.

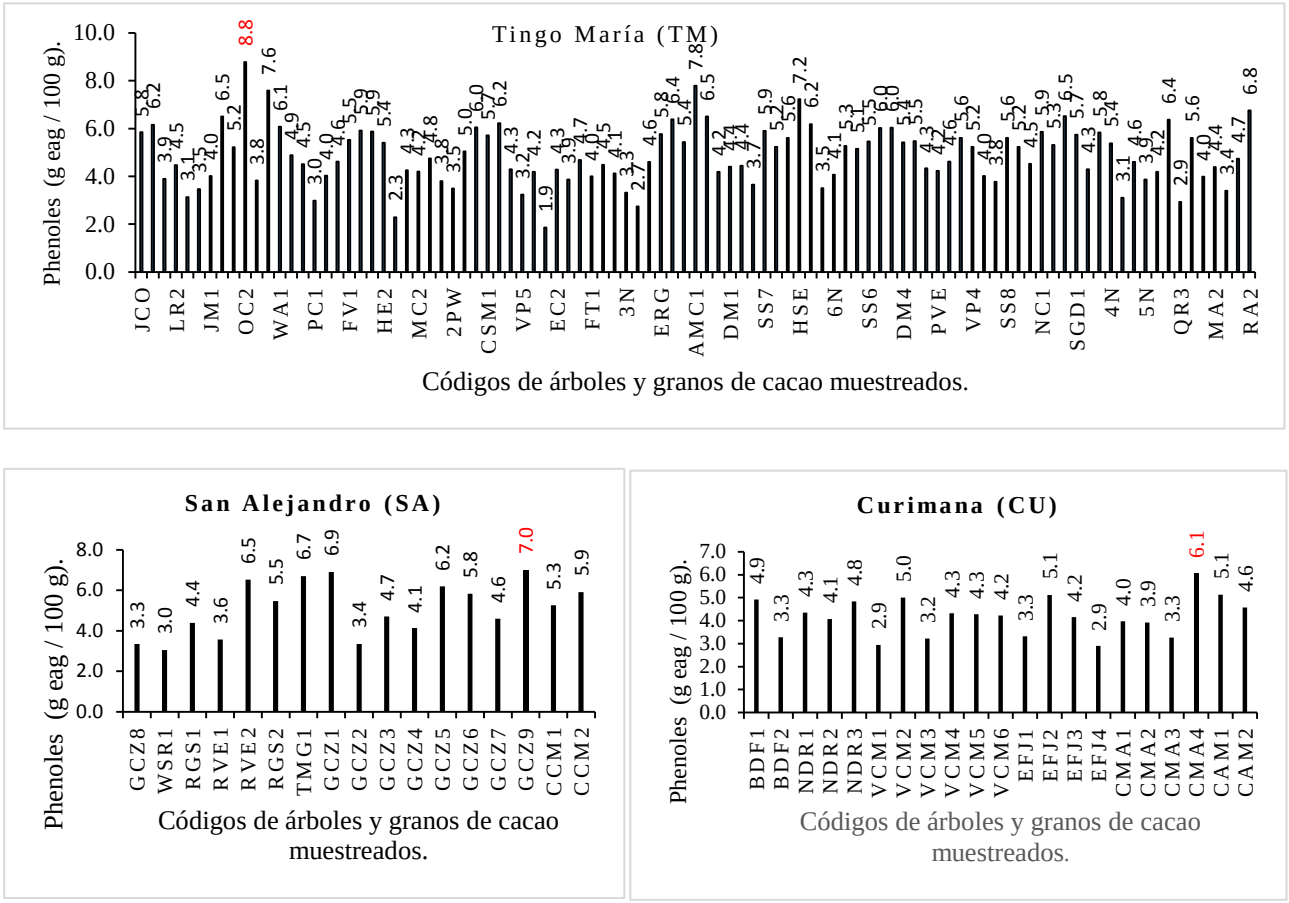


Figure 3. Phenol content in fresh cocoa beans from trees sampled in the production areas of Tingo Maria (TM), San Alejandro (SA), Curimana (CU).

3.2. Obtaining cocoa paste and variation of functional compounds

It was shown that the content of total phenols, anthocyanins, reducing sugars, and the IC_{50} value in samples of fresh cocoa beans, dry fermented grain, in cocoa paste (Table 2), decreased. Because of the effect of fermentation on the chemical characteristics of the grains, likewise it affects these features during the process of making the paste because, in the

process, dry grain were used, roasted, peeled, ground, and went through the conchin, to finally get cocoa paste.

Table 2

Variation in total phenol content, anthocyanins, reducing sugars, and the IC_{50} value in samples of fresh cocoa beans, dried fermented beans, and cocoa paste.

Sample Type	Code	Total phenols (g/100 g of cocoa)	Anthocyanins (mg cyanidin-3-glucoside / g sample)	Reducing sugars (g/L)	DPPH IC_{50}
Fresh grain	OC2	8.77 ± 0.11 ^{f}	91.47 ± 0.17 ^{i}	0.24 ± 0.002 ^{d}	81.22 ± 4.46 ^{abc}
	GCZ9	7.01 ± 0.02 ^{e}	48.71 ± 0.34 ^{g}	0.22 ± 0.003 ^{c}	63.65 ± 0.81 ^{a}
	CMA4	6.07 ± 0.02 ^{d}	44.53 ± 0.91 ^{e}	0.19 ± 0.003 ^{b}	70.06 ± 1.86 ^{b}
Dried fermented grain	OC2	5.92 ± 0.03 ^{d}	43.97 ± 0.23 ^{d}	0.19 ± 0.002 ^{b}	152.10 ± 5.95 ^{e}
	GCZ9	6.11 ± 0.04 ^{d}	43.32 ± 0.17 ^{c}	0.18 ± 0.003 ^{b}	83.60 ± 3.12 ^{abc}
	CMA4	3.40 ± 0.01 ^{b}	44.62 ± 0.68 ^{f}	0.10 ± 0.001 ^{a}	105.40 ± 12.31 ^{cd}
Cocoa paste	OC2	3.43 ± 0.03 ^{b}	37.20 ± 2.84 ^{b}	0.11 ± 0.002 ^{a}	180.24 ± 3.83 ^{e}
	GCZ9	5.33 ± 0.05 ^{c}	28.48 ± 1.88 ^{a}	0.11 ± 0.001 ^{a}	111.79 ± 2.36 ^{d}
	CMA4	2.82 ± 0.03 ^{a}	49.17 ± 1.17 ^{h}	0.10 ± 0.002 ^{a}	165.53 ± 2.94 ^{e}

It is known that in Ghanaian cocoa beans the total polyphenol content, o-diphenols, and anthocyanin content of beans from unstored pods were 18.087 g/100 g, 2.417 g/100 g, and 15.68 mg/kg, respectively. However, the rates of decrease were more dependent on fermentation time than on pod storage (Afoakwa et al., 2012). The total phenol content in fresh grain samples evaluated were 8.77 ± 0.11, 7.01 ± 0.02, 6.07 ± 0.02 g / 100 g of cocoa, and there was a loss of 60.88%, 23.96%, and 53.54%, due to the fermentation and production of cocoa paste. The results of the total phenol content obtained were similar to those reported (Jonfia-Essien et al., 2008), with values of 7-8 g / 100 g. There was a significant difference ($p \leq 0.05$) between the content of phenols in fresh cocoa beans, fermented grain, and cocoa paste.

Table 2 shows that the content of anthocyanins in the fresh beans was 91.47 ± 0.17, 48.71 ± 0.34, 44.53 ± 0.91 mg cyanidin-3-glucoside / g sample, respectively. This content decreased due to the effect of fermentation and the process of obtaining cocoa paste. The loss of anthocyanins coincides with the results obtained by other authors (Bordiga et al., 2015).

Anthocyanins may be degraded through several processes occurring during their extraction, food processing, and storage (Fernandes et al., 2014).

The presence of sugars in the pulp of cocoa beans is important because they serve as a substrate for microorganisms that initiate the fermentation of grain pulp. Fresh cocoa beans are enveloped in a sweet, white, and mucilaginous pulp that represents approximately 40% of raw beans in wet weight (Rodriguez-Campos et al., 2012). The fermentation of cocoa pulp by microorganisms is crucial for developing chocolate flavor precursors. Yeasts conduct alcoholic fermentation within the bean pulp, which is essential for the production of good quality beans, giving typical chocolate characters (Ho et al., 2015). The fermentation and roasting cocoa beans, result in the formation of the characteristic brown grains, phenols, and anthocyanins of raw beans undergo different reactions during fermentation, which leads to the synthesis of flavonols, which contribute to the characteristic brown color of roasted beans. Oxidation and polymerization of phenols, degradation of proteins, Maillard reactions, dextrinization of starch, yield other brown pigments, typical of roasted cocoa (Krysiak, 2006).

3.3. Antioxidant capacity

The antioxidant capacity of the three samples of fresh cocoa beans decreased after fermentation, drying, and in the processing of beans in cocoa paste. When beans are progressively roasted under conditions described as low, medium, and high roast conditions, there is a progressive loss of (-)-epicatechin and (+)-catechin and an increase in (-)-catechin with higher roast levels (Hurst et al., 2011). It has been reported (Ioannone et al., 2015) that the roasting operation increases the antioxidant capacity.

3.4. Principal component analysis

Figure 4 shows the principal component analysis score chart (ACP), which explains the relationship between the samples and the content of total phenols, anthocyanins, reducing sugars, and antioxidant capacity (IC_{50}) while obtaining cocoa paste. The samples of cocoa beans considered were OC2, GCZ9, and CMA4, such as fresh beans, dry fermented beans, and cocoa paste. Components PC-1 and PC-2 explain 100% of the relationships between the samples. In this type of analysis, a lower percentage was reported, which is better when the value is closer to 100% (Tolentino et. Al., 2019). Considering the x-axis, from right to left, it can be seen that the greatest variation in the responses is related to the antioxidant capacity expressed as IC_{50} (in this case, it should be understood that higher values indicate less antioxidant capacity), that is, the antioxidant capacity was higher in the fresh samples, decreasing until having cocoa paste.

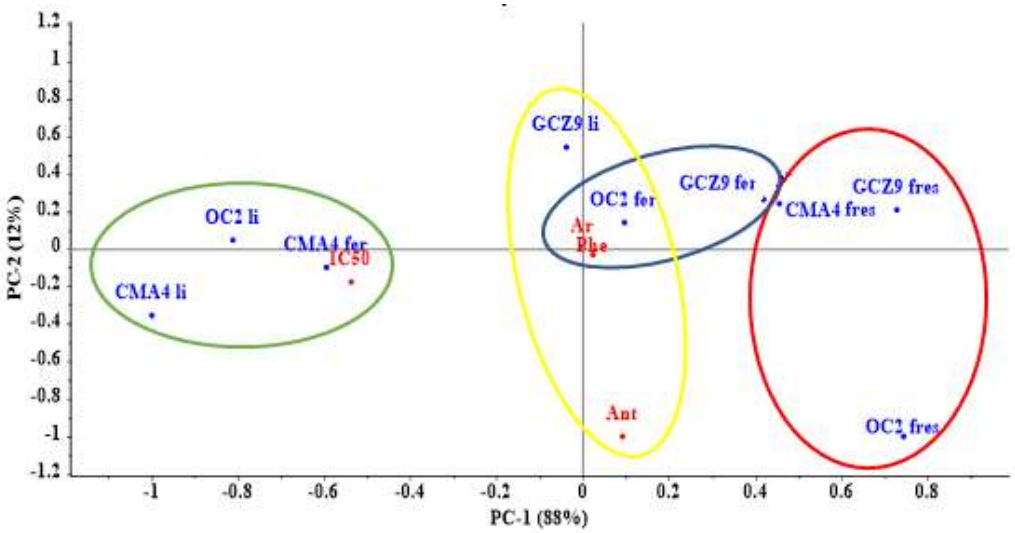


Figure 4. Biplot of Principal component analysis score chart (PCA), PC-1 (88%), and PC-2 (12%), which explain the relationship between the samples and chemical analyses performed while obtaining cocoa paste.

The samples with the highest content of phenols, anthocyanins, reducing sugars, and the highest antioxidant capacity were GCZfres (IC_{50}: 63.65 ± 0.81), CM4fres (IC_{50}: 70.06 ± 1.86), and OC2fres (IC_{50}: 81.22 ± 4.46). As cocoa paste, the OC2 sample was the one with the lowest antioxidant capacity (IC_{50}: 180.24 ± 3.83). The y-axis indicates that the GCZ9li cocoa paste sample had the lowest anthocyanin content, with 28.48 ± 1.88 mg cyanidin-3-glucoside / 1 g sample. Various physiological functions, including antioxidant and antimutagenic activities, are known to have been attributed to polyphenols (Batista el al., 2016).

Figure 5 shows the correlation between the evaluated compounds and the antioxidant capacity (IC50). It can be seen that the antioxidant capacity is mainly influenced by the content of phenols and to a lesser extent by the content of anthocyanins and reducing sugars, decreasing until obtaining a paste of cocoa. During fermentation of cocoa beans, phenols diffuse with cell liquids from their storage cells and are oxidized enzymatically by the polyphenol oxidase to condense high molecular mostly insoluble tannins (Afoakwa et al., 2012).

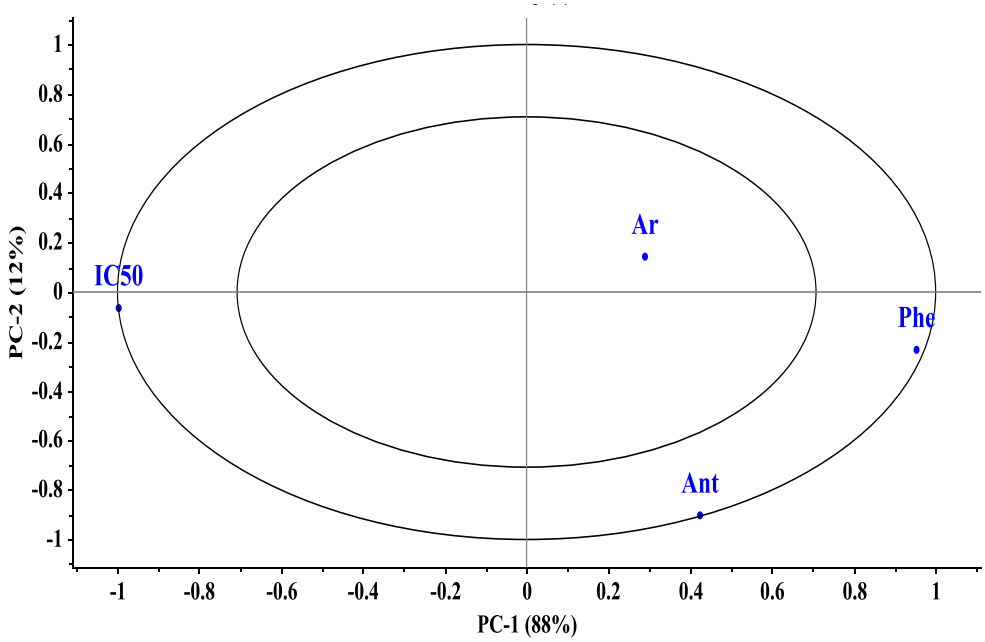
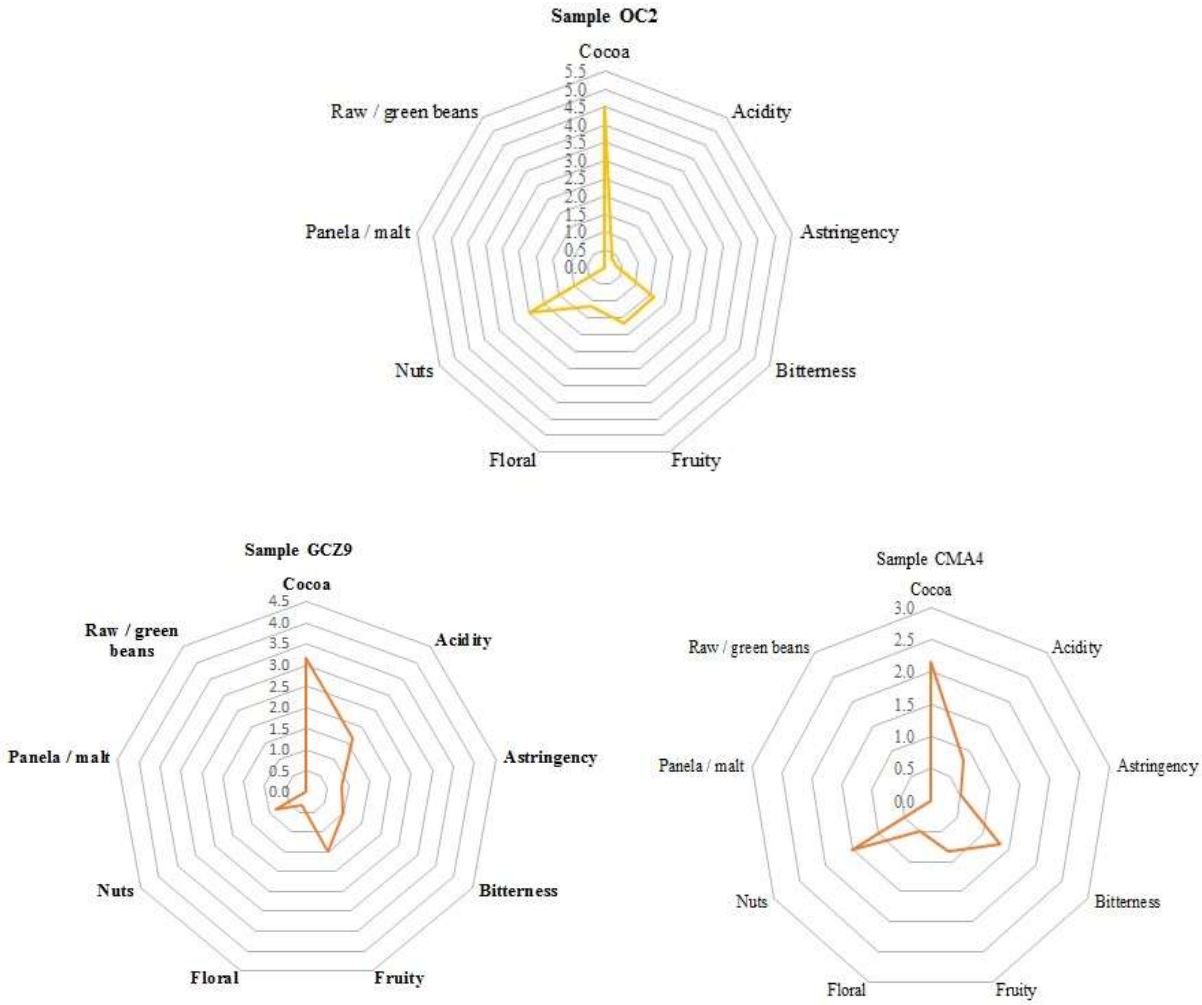


Figure 5. Load correlation between total phenols, anthocinins, reducing sugars, and antioxidant capacity between fresh cocoa beans, dried fermented beans, and cocoa paste.

3.5. Sensory profile of paste

In Figure 6, the scores assigned to each attribute assessed in cocoa paste samples made from grains selected from each area are displayed. The results indicate that sample OC2 is better



suited to the attributes of cocoa, bitterness, fruity, floral, and nuts. The sensory quality of chocolate is widely determined by the qualitative and quantitative composition of volatile

Figure 6. Sensory profile of cocoa paste made with cocoa beans from mother plants, production areas of Tingo Maria (TM - OC2), San Alejandro (SA - GCZ9), and Curimana (CU - CMA4).

compounds resulting from microbial metabolism during fermentation, and Maillard reactions taking place during drying, roasting, and conching (Crafack et al., 2014). More than 600 volatile compounds have been reported to make up a complex mixture that characterizes chocolate aroma, including aldehydes, pyrazines, acids, alcohols, esters, ketones, furans, pyrroles, phenols, terpenes, and terpene alcohols (Ziegleder, 2017).

In Figure 6, the sensory profile of each sample of cocoa paste, made from grains selected from each area, the cocoa paste produced with OC2 shows presented a distinctive flavor of cocoa, along with a nutty flavor in which one can tell the difference. Cocoa paste made with the GCZ9 sample showed a strong cocoa flavor, followed by fruity, with a marked acidity flavor; cocoa paste made with the CMA4 sample had a similar flavor but with less intensity than cocoa paste made with sample OC2. The sensory profiles obtained were similar to those reported in the theory (Crafack et al., 2013). It is also known that after roasting, the bean possesses the typical cocoa aroma and is less astringent, although remaining unpleasant without further processing and addition of ingredients (Misnawi et al., 2004).

4. Conclusions

The content of total phenolic bioactive compounds in fresh cocoa beans differed significantly ($p \leq 0.01$) between samples from different cocoa production areas. The OC2 sample showed 8.8 ± 0.1 g EAG / 100 g of grain from the TM zone; GCZ9 sample showed 7.0 ± 0.02 g EAG / 100 g of grain from the SA zone; CMA4 sample showed 6.1 ± 0.02 g EAG / 100 g grain from the CU zone. The total phenol content of the selected fresh cocoa beans decreased by 60.88%, 23.96%, and 53.54%, respectively.

The multivariate analysis indicated that fresh cocoa beans had the highest antioxidant capacity and were GCZfres (IC_{50}: 63.65 ± 0.81), CM4fres (IC_{50}: 70.06 ± 1.86), and OC2fres (IC_{50}: 81.22 ± 4.46). As cocoa paste, the OC2 sample was the one with the lowest antioxidant capacity (IC_{50}: 180.24 ± 3.83). In also turned out that the GCZ9li cocoa paste sample had the lowest anthocyanin content (28.48 ± 1.88 mg cyanidin-3-glucoside / 1 g sample).

Correlation analysis determined that the antioxidant capacity was mainly influenced by the content of phenols and, to a lesser extent, by the content of anthocyanins and reducing sugars.

OC2 cocoa paste had a distinctive cocoa flavor, GCZ9 paste showed a strong cocoa flavor, followed by fruity, with marked acidity, and CMA4 paste had a similar flavor but with less intensity than OC2.

Acknowledgments: The authors would like to thank the Fund for Innovation, Science and Technology (FINCyT), Ministry of Production of Peru, for research funding.

Bibliographic references

Ackar, D., Valek Lendić, K., Valek, M., Subarić, D., Miličević, B., Babić, J., Nedić, I. (2013). *Cocoa polyphenols: Can we Consider cocoa and chocolate as potential functional Food?* Journal of Chemistry, 2013, 1–7. http://dx.doi.org/10.1155/2013/289392

Afoakwa, E. O., Quao, J., Takrama, F. S., Budu, A. S., Saalia, F. K. (2012). *Changes in total polyphenols, o-diphenols, and anthocyanin concentrations during fermentation of pulp pre-conditioned cocoa (Theobroma cacao) beans*. International Food Research Journal, 19(3), 1071-1077. Retrieved from http://search.proquest.com/docview/1315524574?accountid=170233

AOAC. (1995). *Official methods of analysis of AOAC international; agricultural chemicals, foods, contaminants, and drugs*. ISED Gaithersburg Md. USA AOAC International. 1141 p.

Arias, L.D., Montaño, D.L.N., Velasco, S. M.A., Martínez, G. J. (2018). *Alimentos funcionales: avances de aplicación en agroindustria*. Revista Tecnura, *22*(57), 55-68. https://doi.org/10.14483/22487638.12178

Avello, M., Suwalski, M. (2006). *Radicales Libres, antioxidantes naturales y mecanismo de protección*, Atenea 464, II Seminario. pp. 161.

Batista, N. N., de Andrade, D. P., Ramos, C. L., Dias, D. R., Schwan, R. F. (2016). *Antioxidant capacity of cocoa beans and chocolate assessed by FTIR*. Food Research International, 90, 313–319. https://doi.org/10.1016/j.foodres.2016.10.028

Bordiga, M., Locatelli, M., Travaglia, F., Coïsson, J. D., Mazza, G., Arlorio, M. (2015). *Evaluation of the effect of processing on cocoa polyphenols: antiradical activity, anthocyanins, and procyanidins profiling from raw beans to chocolate*. International Journal of Food Science & Technology, 50(3), 840–848. http://dx.doi.org/10.1111/ijfs.12760

Brand-Williams W., Cuvelier, M.E. y Berset, C. (1995). *Use of free radical method to evaluate antioxidant activity*. LWT - Food Sci. Technol., Massy. 28(1): 25:30.

Crafack, M., Keul, H., Eskildsen, C. E., Petersen, M. A., Saerens, S., Blennow, A., Nielsen, D. S. (2014). *Impact of starter cultures and fermentation techniques on the volatile aroma and sensory profile of chocolate*. Food Research International, 63, 306–316. http://dx.doi.org/10.1016/j.foodres.2014.04.032

Crafack, M., Mikkelsen, M. B., Saerens, S., Knudsen, M., Blennow, A., Lowor, S., Nielsen, D. S. (2013). *Influencing cocoa flavour using Pichia kluyveri and Kluyveromyces marxianus in a defined mixed starter culture for cocoa fermentation*. International

Journal of Food Microbiology, 167(1), 103–116. http://dx.doi.org/10.1016/j.ijfoodmicro.2013.06.024

Daza P. J.F. (2006). *Estadística aplicada con Microsoft Excel*. Ed. Megabyte S.A.C. Lima, Perú. p. 485 – 516.

Di Carro, M., Ardini, F., Magi, E. (2015). *Multivariate optimization of headspace solid-phase microextraction followed by gas chromatography–mass spectrometry for the determination of methylpyrazines in cocoa liquors.* Microchemical Journal, 121, 172–177. http://dx.doi.org/10.1016/j.microc.2015.03.006

Diaz S., Pinoargote, M. (2012). *Análisis de las Características Organolépticas del Chocolate a partir de Cacao CCN51 Tratado Enzimáticamente y Tostado a Diferentes Temperaturas.* Escuela Superior Politécnica Del Litoral**.** Guayaquil – Ecuador. 97p

Dostert, N., Roque, J., Cano, A., La Torre, M., Weigend, M. (2012). *Hoja botánica: Cacao, Theobroma cacao L.* Impreso por: Giacomotti Comunicación Gráfica S.A.C. Primera edición.Lima, Perú. 20 p.

Esbensen, K.; Swarbrick, B. (2017). *Multivariate Data Analysis*. 6th Edition. Copenhagen, Sydney, Oslo, EEUU. Pp. 1-452.

Fernandes, I., Faria, A., Calhau, C., De Freitas, V., Mateus, N. (2014). *Bioavailability of anthocyanins and derivatives.* Journal of Functional Foods, 7, 54–66. http://dx.doi.org/10.1016/j.jff.2013.05.010

García Bolívar, J. J., Mina Ortega, J. I., Torres Mayanquer, F., Burbano Pulles, M., & Yambay Vallejo, W. J. (2017). *Evaluación sensorial y metodologías para su análisis*. doi:10.32645/9789942914439

Gil, J. (2012). *Estabilidad y actividad antioxidante de catequinas presentes en cacaos colombianos durante los procesos de pre e industrialización*. Medellín, Colombia. Universidad de Antioquía. 119p.

Gusakov, V., Kondratyeva E., Sinitsyn A. (2011). *Comparison of Two Methods for Assaying Reducing Sugars in the Determination of Carbohydrase Activities.* International Journal of Analytical ChemistryVolume2011, Article ID 283658, 4 p. doi: https://doi.org/10.1155/2011/283658

Ho, V. T. T., Zhao, J., Fleet, G. (2015). *The effect of lactic acid bacteria on cocoa bean fermentation.* International Journal of Food Microbiology, 205, 54–67. http://dx.doi.org/10.1016/j.ijfoodmicro.2015.03.031

Hurst, W. J., Krake, S. H., Bergmeier, S. C., Payne, M. J., Miller, K. B., Stuart, D. A. (2011). *Impact of fermentation, drying, roasting, and Dutch processing on flavan-3-ol*

stereochemistry in cacao beans and cocoa ingredients. Chemistry Central Journal, 5(1), 53. http://dx.doi.org/10.1186/1752-153x-5-53

Ioannone, F., Di Mattia, C. D., De Gregorio, M., Sergi, M., Serafini, M., Sacchetti, G. (2015). *Flavanols, proanthocyanidins, and antioxidant activity changes during cocoa (Theobroma cacao L.) roasting as affected by temperature and time of processing.* Food Chemistry, 174, 256–262. http://dx.doi.org/10.1016/j.foodchem.2014.11.019

John, W. A., Böttcher, N. L., Aßkamp, M., Bergounhou, A., Kumari, N., Ho, P.-W., … Ullrich, M. S. (2019). *Forcing fermentation: Profiling proteins, peptides, and polyphenols in lab-scale cocoa bean fermentation*. Food Chemistry, 278, 786–794. doi:10.1016/j.foodchem.2018.11.108

Jonfia-Essien, W. A., West, G., Alderson, P. G., Tucker, G. (2008). *Phenolic content and antioxidant capacity of hybrid variety cocoa beans.* Food Chemistry, 108(3), 1155–1159. http://dx.doi.org/10.1016/j.foodchem.2007.12.001

Krysiak, W. (2006). *Influence of roasting conditions on coloration of roasted cocoa beans.* Journal of Food Engineering, 77(3), 449–453. http://dx.doi.org/10.1016/j.jfoodeng.2005.07.013

López B. E.A. (2008). *Diseño y análisis de experimentos, fundamentos y aplicaciones en agronomía*. Universidad de San Carlos de Guatemala. Guatemala. 176 p.

Martin, M. A., Goya, L., Ramos, S. (2013). *Potential for preventive effects of cocoa and cocoa polyphenols in cancer*. Food and Chemical Toxicology, 56, 336–351. http://dx.doi.org/10.1016/j.fct.2013.02.020

Mata, D. (2018). *Proceso de comercialización del cacao fino de Aroma en la provincia Los Ríos, Ecuador*. Avances, 20(4), 385-400. Retrieved from http://www.ciget.pinar.cu/ojs/index.php/publicaciones/article/view/389/1354

Mesa, L.; Gozá, O.; Uranga, M.; Toledo, A.; Gálvez Y. (2018). *Aplicación del Análisis de Componentes Principales en el proceso de fermentación de un anticuerpo monoclonal*. Vaccimonitor. Cuba 27(1): 8-15.

Miller, G. (1959). *Use of dinitrosalicylic acid reagent for determination of reducing sugar*. Analytical Chemistry, vol.31, no.3, p. 426 – 428. doi: https://doi.org/10.1021/ac60147a030.

Misnawi, Jinap, S., Jamilah, B., Nazamid, S. (2004). *Sensory properties of cocoa liquor as affected by polyphenol concentration and duration of roasting.* Food Quality and Preference, 15(5), 403–409. http://dx.doi.org/10.1016/s0950-3293(03)00097-1

NTP 208.040. (2008). *Buenas prácticas para la cosecha y beneficio del cacao*.

Ooi, T. S., Ting, A. S. Y., Siow, L. F. (2020). *Influence of selected native yeast starter cultures on the antioxidant activities, fermentation index and total soluble solids of Malaysia cocoa beans: A simulation study*. LWT, 122, 108977. doi:10.1016/j.lwt.2019.108977

Peláez, P., Bardón, I., Camasca, P. (2016). *Methylxanthine and catechin content of fresh and fermented cocoa beans, dried cocoa beans, and cocoa liquor*. Scientia Agropecuaria, 7, 355–365. doi:10.17268/sci.agropecu.2016.04.01

Poo, B. (2005). *Concentración de Antocianinas en jugo de cranberries (VacciniummacrocarponAit.) mediante Nano filtración. Universidad Austral de Chile.* Facultad de Ciencias Agrarias. Escuela de Ingeniería en alimentos. Valdivia –Chile. [En línea]: http://cybertesis.uach.cl/tesis /uch/2005/fap821c/doc/fap821c.pdf

Portillo, E., Graziani, L., Betancourt, E. (2007). *Análisis químico del cacao criollo porcelana (Theobroma cacao L.) en el Sur del lago de Maracaibo.* Revista Fac. Agron. (LUZ). 24:522-546.

Ramos, F., Muñoz, A., Ortiz, C., Yañez, J. (2010). *Antocianinas, polifenoles, actividad anti-oxidante de sachapapa morada (Dioscorea trífida L.) y evaluación de lipoperoxidacion en suero humano.* Rev.Soc.Quim. Perú. 76 (1): 61 – 72.

Rodriguez-Campos, J., Escalona-Buendía, H. B., Contreras-Ramos, S. M., Orozco-Avila, I., Jaramillo-Flores, E., Lugo-Cervantes, E. (2012). *Effect of fermentation time and drying temperature on volatile compounds in cocoa.* Food Chemistry, 132(1), 277–288. http://dx.doi.org/10.1016/j.foodchem.2011.10.078

Romaina J.C. (2012). Estadística experimental, herramientas para investigación. Fondo editorial Universidad Privada de Tacna. [en línea]: http://www.iiap.org.pe/upload/Transparencia/Actualizaciones%202011-2013/TRANSP632/20130129/CursoEstadistica/TEXTOS/estadisticaexperimental.pdf

Schinella, G., Mosca, S., Cienfuegos-Jovellanos, E., Pasamar, M. Á., Muguerza, B., Ramón, D., Ríos, J. L. (2010). *Antioxidant properties of polyphenol-rich cocoa products industrially processed.* Food Research International, 43(6), 1614–1623. http://dx.doi.org/10.1002/9781118229378.ch1

Suazo, Y. (2012). *Efecto de la fermentación y el tostado sobre la concentración polifenólica y actividad antioxidante de cacao nicaragüense.* Nicaragua. Universidad Pública De Navarra. 62 p.

Sultana, B., Hussain, Z., Asif, M.; Munir, A. (2012). *Investigation on the Antioxidant Activity of Leaves, Peels, Stems Bark, and Kernel of Mango (Mangiferaindica L.).* J Food Sci 77(8): C849-52.

Symonowicz, M., Sykula-Zajac, A., Lodyga-Chruścińska, E., Rumora, I., Straukas, M. (2012). *Evaluation of polyphenols and anthocyanins contents in black chockeberry - Photiniamelanocarpa (Michx.) fruits extract.* Acta Pol Pharm69 (3): 381-7.

Tardzenyuy, M. E., Jianguo, Z., Akyene, T., Mbuwel, M. P. (2020). *Improving cocoa beans value chain using a local convection dryer: A case study of Fako division Cameroon*. *Scientific African, 8*, e00343. https://doi.org/10.1016/j.sciaf.2020.e00343

Tolentino, M. E., Camasca, P., Peláez, P. P. (2019). *Macro and microelements, lead, cadmium, functional compounds, antioxidant capacity in fresh, dry cocoa beans and cocoa paste*. Scientia Agropecuaria, 10(4), 521–530. https://doi.org/10.17268/sci.agropecu.2019.04.09

Torres-Moreno, M., Torrescasana, E., Salas-Salvadó, J., Blanch, C. (2015). *Nutritional composition and fatty acids profile in cocoa beans and chocolates with different geographical origin and processing conditions.* Food Chemistry, 166, 125–132. http://dx.doi.org/10.1016/j.foodchem.2014.05.141

Ziegleder, G. (2017). *Flavour development in cocoa and chocolate*. Beckett's Industrial Chocolate Manufacture and Use, 185–215. doi:10.1002/9781118923597.ch8

Żyżelewicz, D., Budryn, G., Oracz, J., Antolak, H., Kręgiel, D., Kaczmarska, M. (2018). *The effect on bioactive components and characteristics of chocolate by functionalization with raw cocoa beans*. Food Research International, 113, 234–244. doi:10.1016/j.foodres.2018.07.017